U.S. Department of Health and Human Services

National Institutes of Health

National Heart, Lung, and Blood Institute

# BIOCHEMICAL MECHANISMS OF DRUG TOXICITIES

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# TYPES OF ADRs

Cardiac
Skin
Renal
Pulmonary
Neurological
Lupus
Hepatic

Anaphylaxis
Hemolytic anemia
Granulocytopenia
Thrombocytopenia
Aplastic anemia
Vasculitis

# SEVERITY OF ADRs

* Minor
* Severe (SADRs)
  - 6.2-6.7% hospitalized patients in USA
  - over 2 million hospitalized patients
  - 100,000 deaths
  - similar findings in Europe and Australia
  - tens of billions of dollars cost burden

*Wilke, et al., Nature Review-Drug Discovery, 904, 2007*

# LEADING CAUSES OF DEATH IN USA IN 1994

| | |
|---|---|
| Heart disease | 743,460 |
| Cancer | 529,904 |
| Stroke | 150,108 |
| SADRs | 106,000 |
| Pulmonary disease | 101,077 |
| Accidents | 90,523 |
| Pneumonia | 75,719 |
| Diabetes | 53,894 |

*Lazarou et al., JAMA, 279, 1208 (1998)*

# TOXICITIES LEADING TO DRUG WITHDRAWAL 1976-2005 IN USA

* **Hepatoxicity, 6 (21%)**
* **Torsades, 6 (21%)**
* **Cardiotoxicity, 2 (7%)**
* **Nephrotoxicity, 2 (7%)**
* **Rhabdomyolyis, 2 (7%)**
* **Others, 10 (37%)**

***Wilke, et al., Nature Review-Drug Discovery, 904, 2007***

## TYPE A ADRs

* 80% of ADRs
* Occur frequently
* Dose-dependent
* Excessive or diminished pharmacologic effects
* Drug-drug interactions
* Often uncovered preclinically

*Endres, et al., European Journal of Pharmaceutical Sciences, 27, 501 (2006)*

## EXAMPLES OF TYPE A ADRS

* Drowsiness from antihistamines
* Hypotension from anti-hypertensive therapy
* Excess bleeding from warfarin
* Posicor
* Acetaminophen

# TYPE B ADRs

* 20% of ADRs
* Rare, unpredictable, and highly host-dependent
* Mechanisms often unknown
  - allergic reactions
  - pseudoallergic reactions
  - dysregulation of signalling pathways
  - genetic and environmental factors
* Rarely reproduced in animals

# SADRs CAUSED BY DRUG METABOLIZING ENZYME POLYMORPHISMS

* Anti-malarial- and sulfonamide-induced hemolytic anemia caused by low G6PD
* Sensitivity to warfarin by CYP2C9*2 and *3
* Irinotecan fatal diarrhea and neutropenia caused by UGT1A1*28 and other allelic forms of UGT1A
* Prolonged neuromuscular blockade by serum choline esterase deficiency

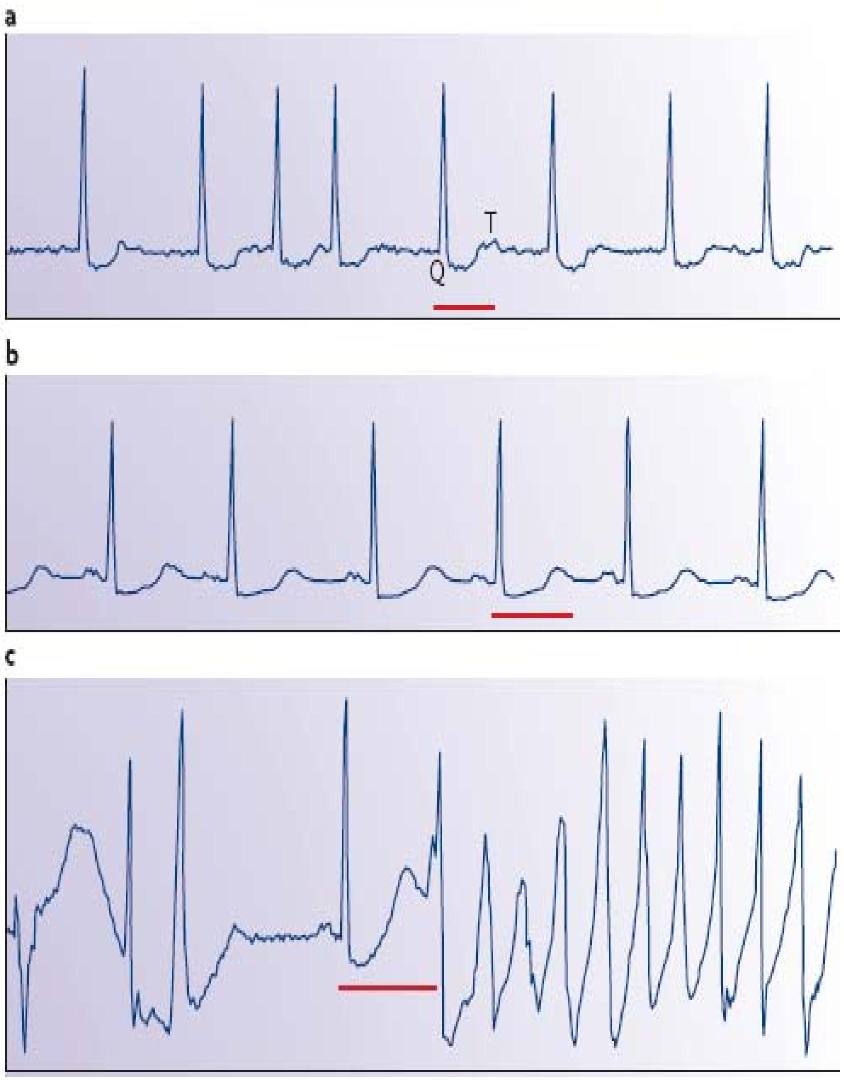
*Reider, et al., N.E.J.M., 352, 2285 (2005); Han et al., J. Clin. Oncol., 24, 2237 -2244 (2006); Barta, et al., Mol.Genet.Metab., 74, 484 (2001)*

# SADRs CAUSED BY DRUG TARGET POLYMORPHISMS

* Warfarin resistance due to vitamin K oxide reductase complex subunit 1 (VKORC1) overexpression

*Reider, et al., N.E.J.M., 352, 2285 (2005)*

# DRUG-INDUCED-LONG QT SYNDROME AND TORSADES DE POINTES




***Nature Review-Drug Discovery, 904, 2007***

# DRUG-INDUCED-LONG QT SYNDROME

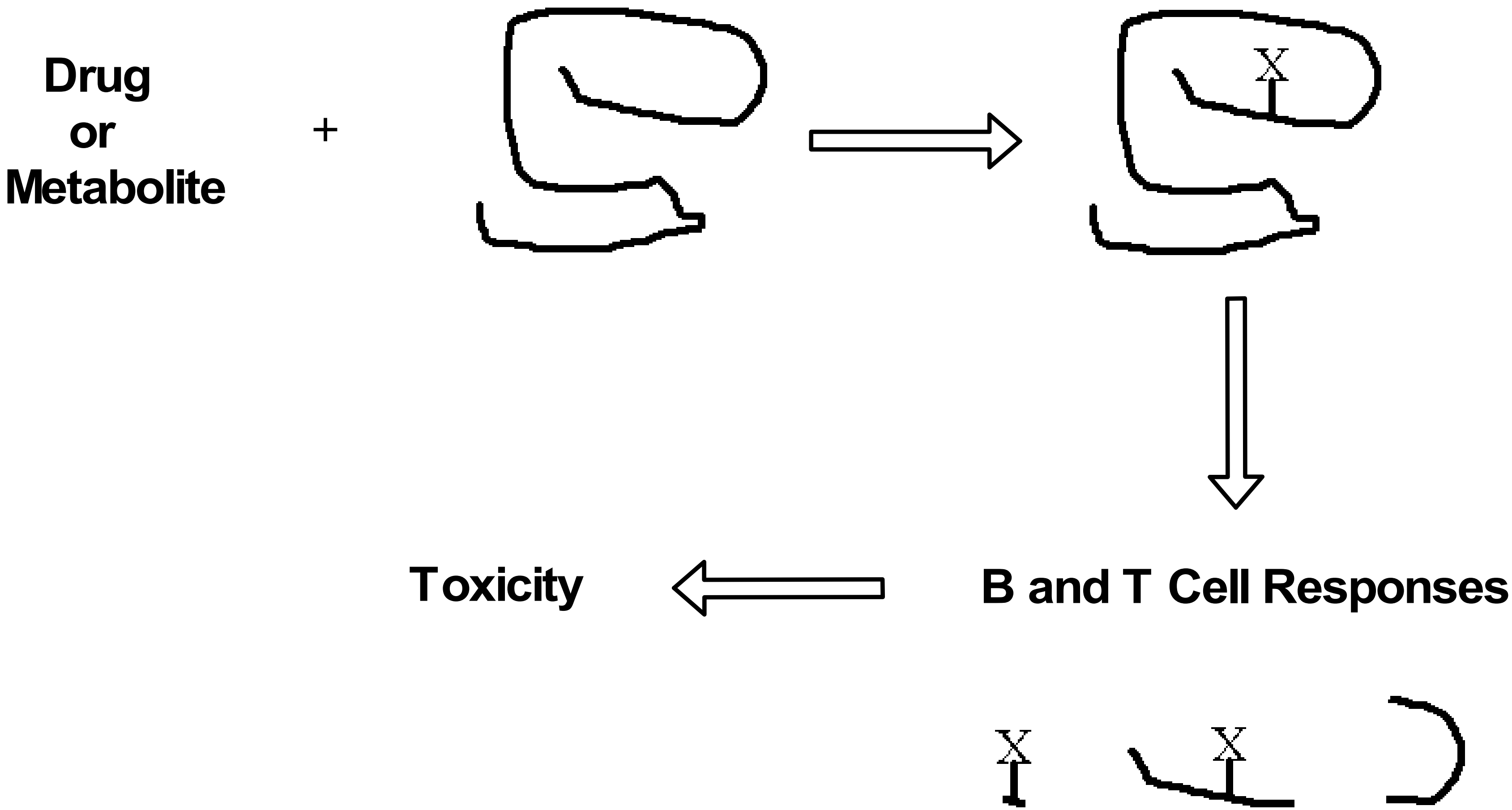
* Structurally diverse drugs cause DLQTS including antiarrhythmics, antihistamines, antipsychotics, antibiotics and others
* Several withdrawn from the market place including Seldane and Propulsid
* Blockage of the cardiac potassium channel hERG
* Inhibition of hERG channel trafficking to plasma membrane

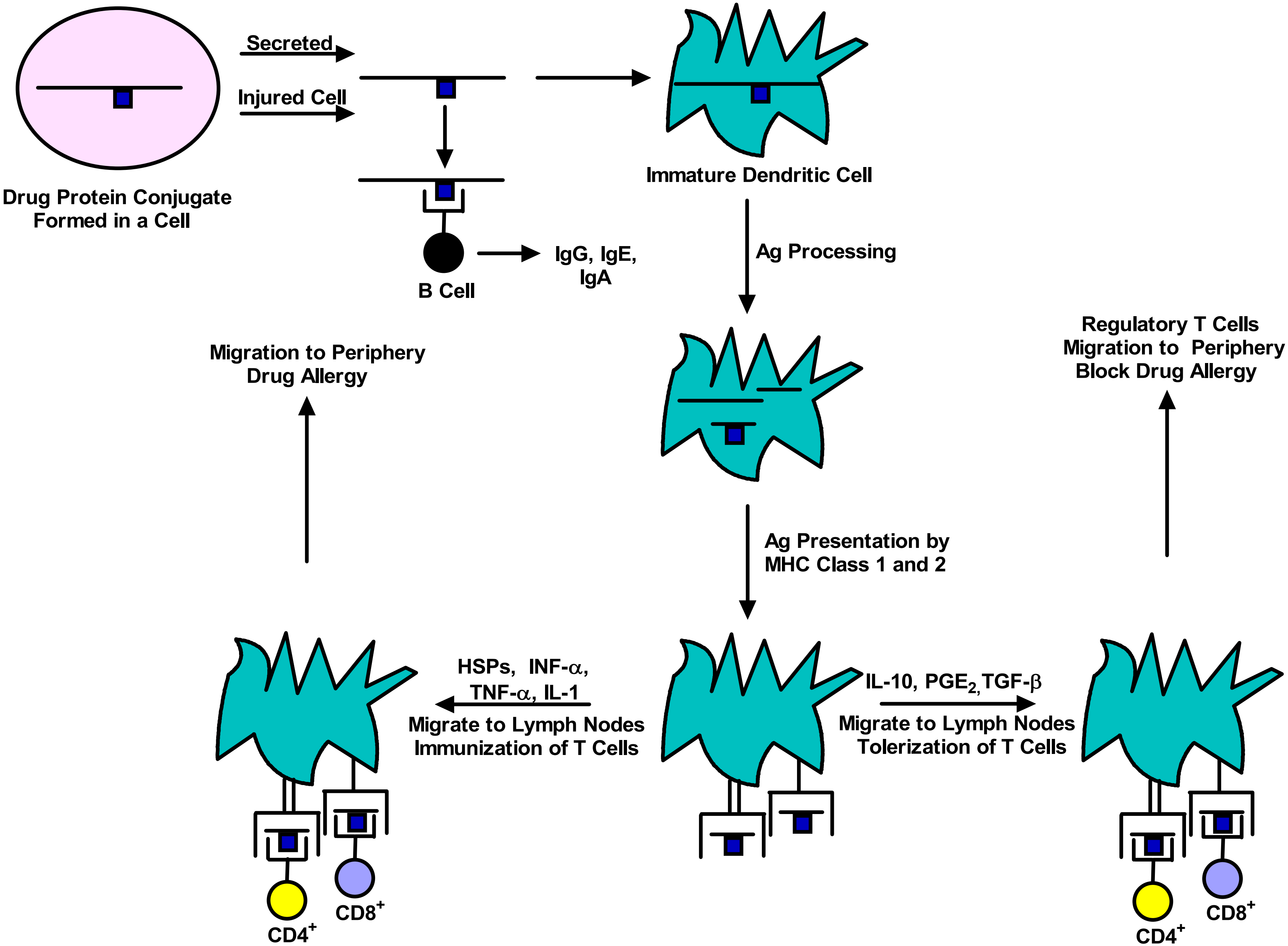
# DRUG-INDUCED-LONG QT SYNDROME

* *In vitro* molecular, cellular, and tissue assays have been developed to measure these interactions

*Dennis, et al., Biochemical Society Transactions, 35, 1060 (2007); Meyer, et al., Expert Opin. Drug Metab. Toxicol., 3, 507 (2007)*

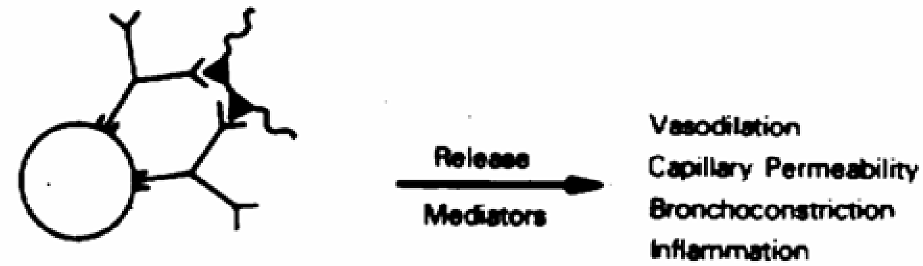
# HAPTEN HYPOTHESIS AND DRUG-INDUCED ALLERGIC REACTIONS




Secreted
Injured Cell
Drug Protein Conjugate
Formed in a Cell
Immature Dendritic Cell
B Cell
IgG, IgE,
IgA
Ag Processing
Regulatory T Cells
Migration to Periphery
Block Drug Allergy
Migration to Periphery
Drug Allergy
Ag Presentation by
MHC Class 1 and 2
HSPs, INF-α,
TNF-α, IL-1
Migrate to Lymph Nodes
Immunization of T Cells
IL-10, PGE2, TGF-β
Migrate to Lymph Nodes
Tolerization of T Cells
CD4+
CD8+
CD4+
CD8+

# PATHWAYS OF IMMUNOPATHOLOGY

A.

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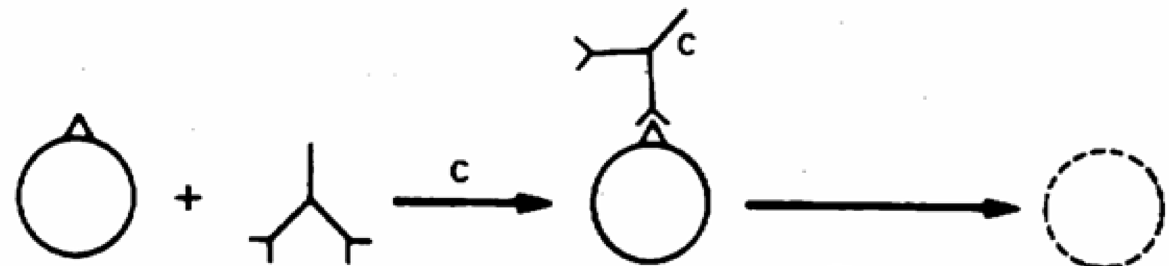
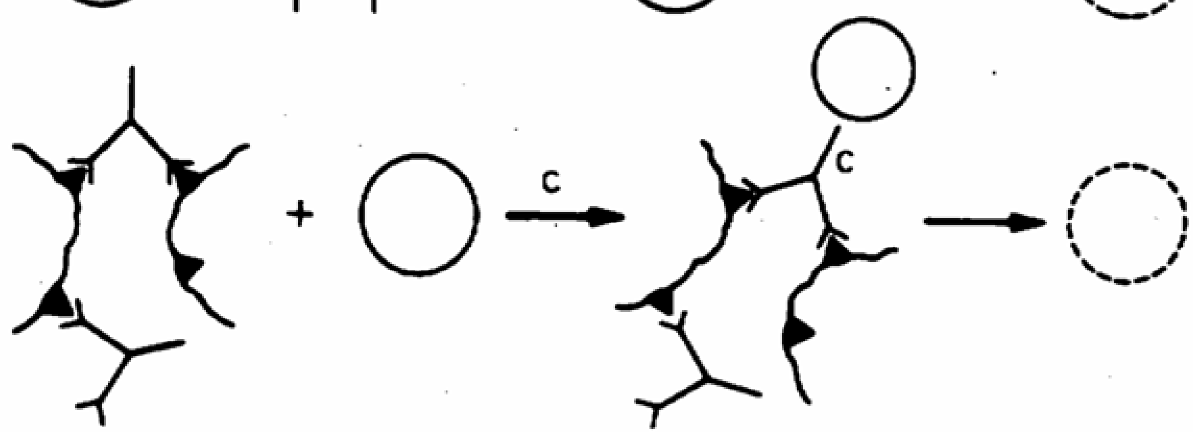
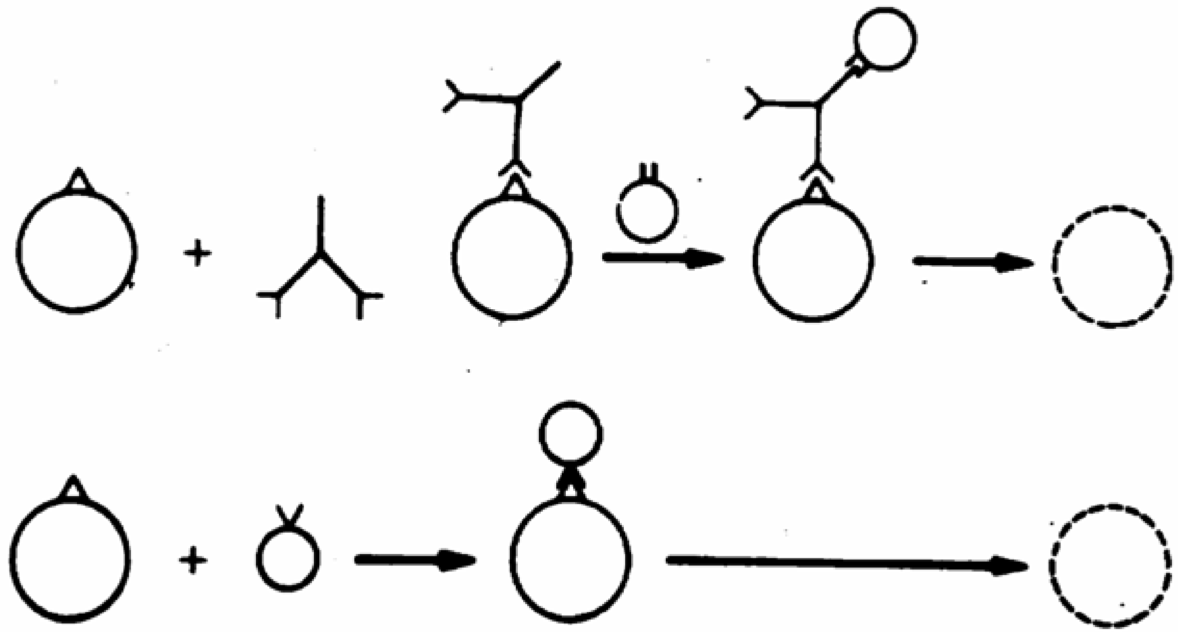



C.



D.

E.



# CUTANEOUS DRUG REACTIONS

* 95% are self-limiting rashes
* SJS and TEN can be life-threatening with blisters, skin detachment, and mucosa involvement
* Most appear to be immune-mediated by drug-specific IgE antibodies while many others by $CD4^+$ and $CD8^+$ T cells

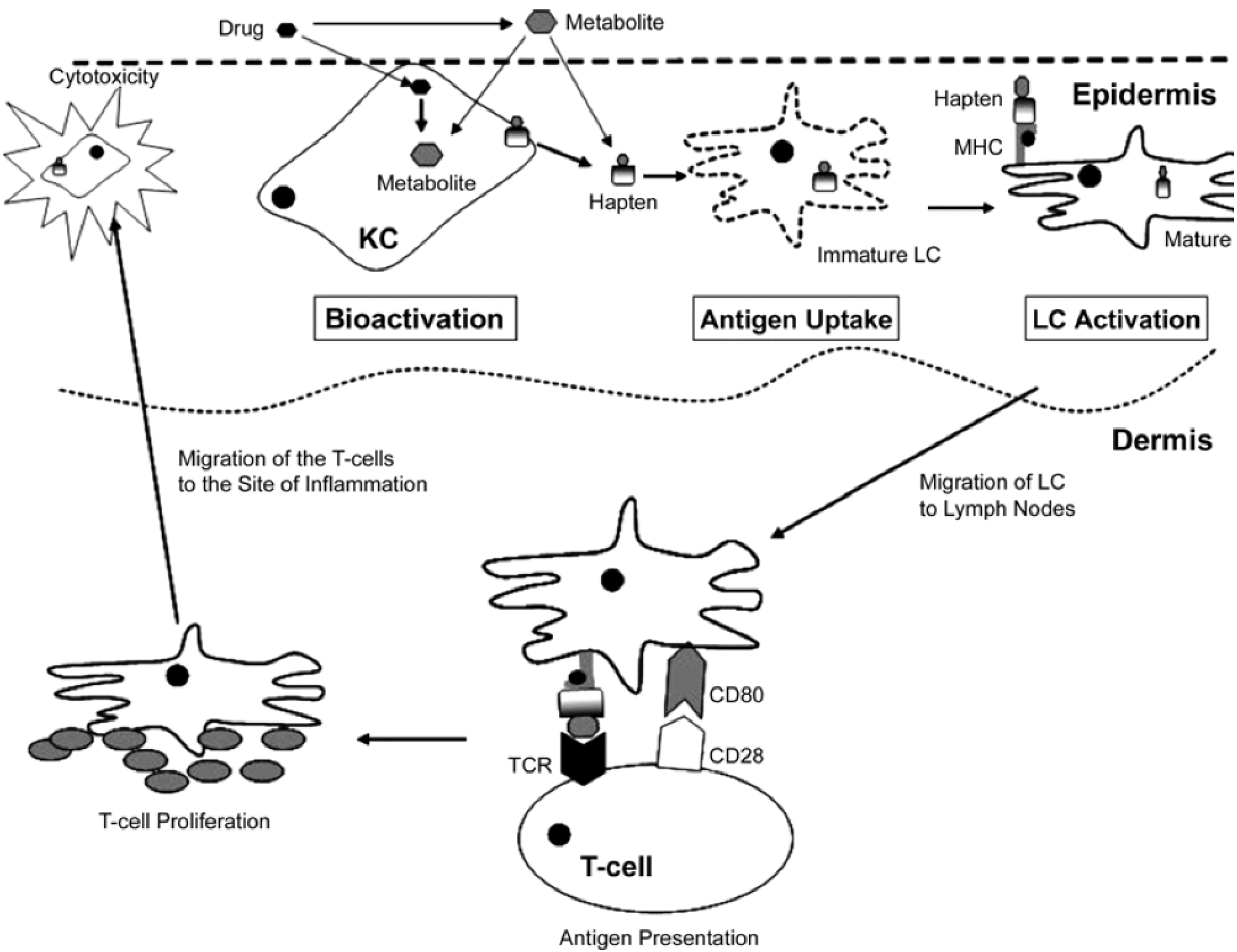
*Roychowdhury and Svensson, AAPS J., 7, E 434 (2005)*

# MACULO-PAPULAR EXANTHEM AND TOXIC EPIDERMAL NECROLYSIS


# T CELL REACTIVITY TO DRUGS CAUSING CUTANEOUS ADRS

- **Lidocaine**
- **Sulfonamides**
- **β-Lactam antibiotics**
- **Phenytoin**
- **Carbamazepine**

***Lebrec et al., Cell Biology and Toxicology, 15, 57 (1999); Naisbitt, et al., Expert Opin. Drug Saf., 6, 109 (2007); Posadas and Pichler, Clin. Experimental Allergy, 37, 989 (2007)***


Drug
Metabolite
Cytotoxicity
Metabolite
KC
Hapten
Immature LC
Hapten
MHC
Epidermis
Mature
Bioactivation
Antigen Uptake
LC Activation
Dermis
Migration of the T-cells
to the Site of Inflammation
Migration of LC
to Lymph Nodes
CD80
CD28
TCR
T-cell
T-cell Proliferation
Antigen Presentation

# HLA-B*1502 ASSOCIATED WITH CBZ-INDUCED SJS/TEN

* Seen in south-east Asians but not in Caucasians
* 98.3% (59/60) CBZ-SJS/TEN positive
* 4.2% (6/144) CBZ-tolerant positive
* High sensitivity/specificity of this test can be used to screen patients receiving CBZ

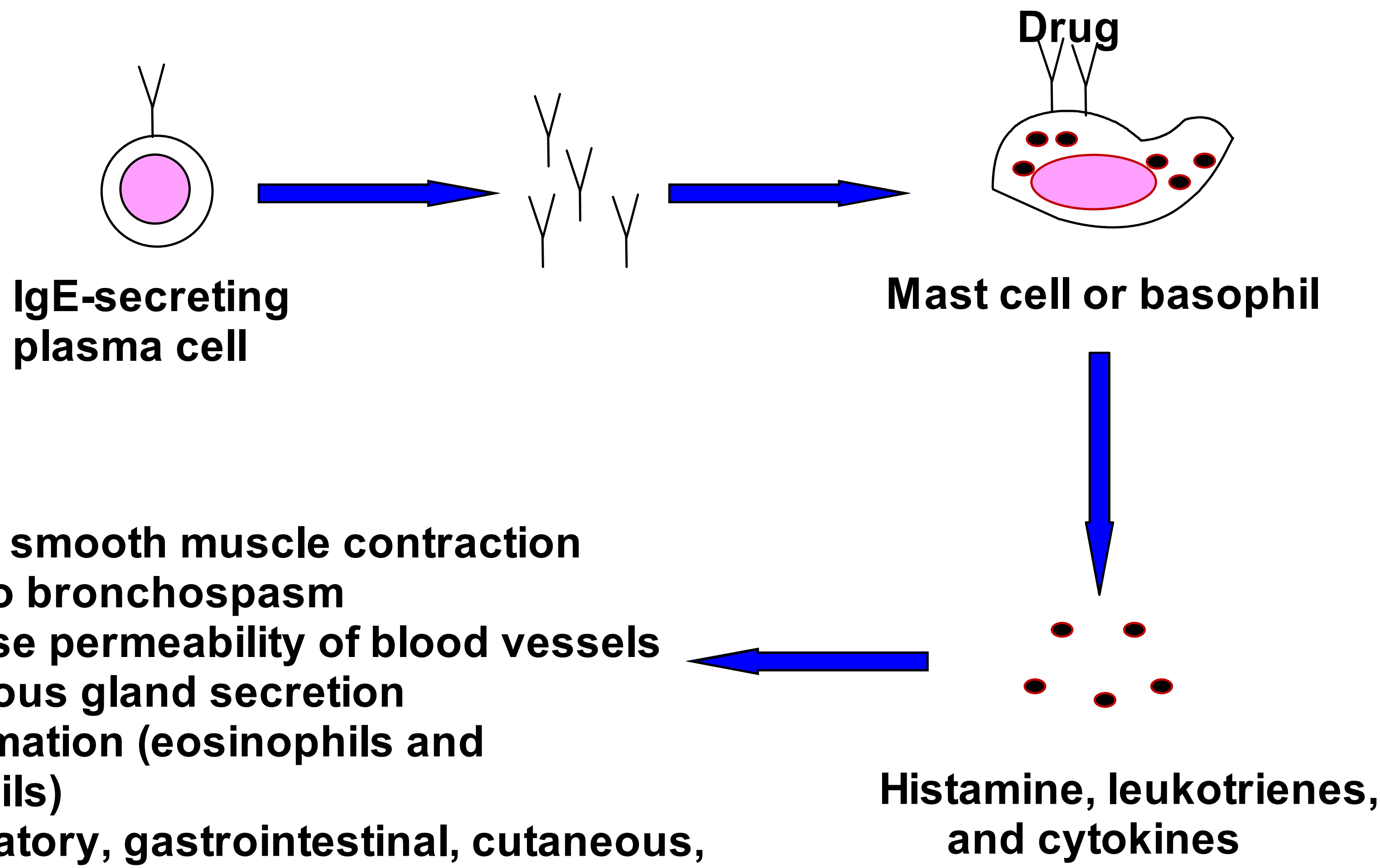
*Chung, et al., Curr. Opin. Allergy Clin. Immunol., 7, 317 (2007)*

# IgE-MEDIATED ANAPHYLACTIC DRUG REACTIONS

| | |
|---|---|
| **Alcuronium** | **Sulfamethoxazole** |
| **Cephalosporins** | **Suxamethonium** |
| **Penicillins** | **Thiopentone** |
| **Protamine** | **Trimethoprine** |
| **Streptokinase** | **Tubocurarine** |

*Park et al., Chem. Res. Toxicol., 11, 969 (1998);Thong and Chan, Ann. Allergy Asthma Immunol., 92, 619 (2004)*

# MECHANISM OF DRUG-INDUCED ANAPHYLAXIS




1. Airway smooth muscle contraction leading to bronchospasm
2. Increase permeability of blood vessels and mucous gland secretion
3. Inflammation (eosinophils and neutrophils)
4. Respiratory, gastrointestinal, cutaneous, and cardiovascular systems can be involved

# DRUG-INDUCED LIVER DISEASE IS A MAJOR HEALTH PROBLEM

It is a major cause of acute liver failure and a major safety reason for:

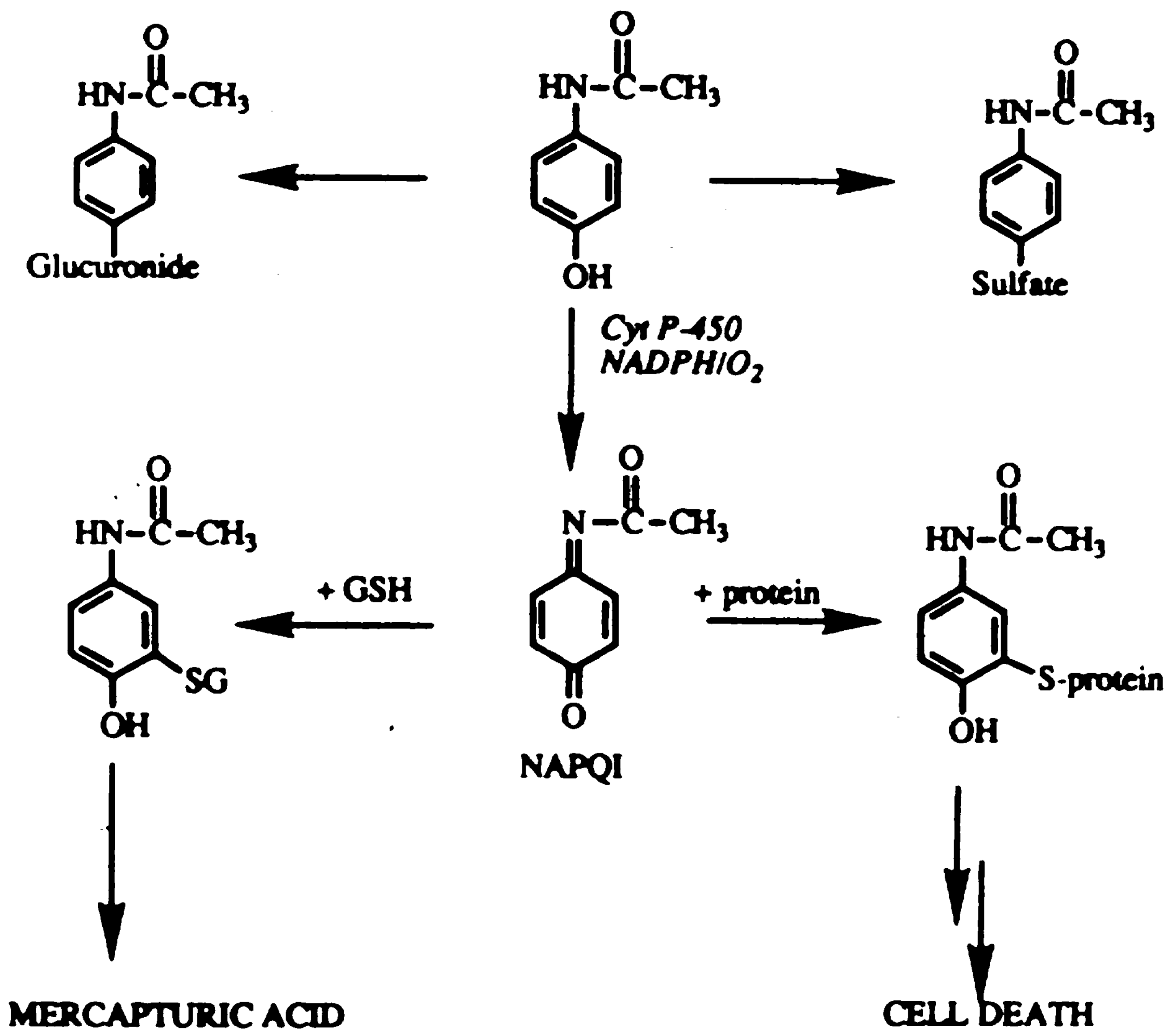
* Stopping preclinical development of drugs
* Terminating clinical trials of drugs
* Withdrawing drugs postmarketing

F. Ballet, J. Hepatol., 26 (Suppl. 2), 26 (1997)

# DRUGS WITHDRAWN / NOT APPROVED DUE TO LIVER DISEASE

| | |
|---|---|
| Iproniazid | 1956 |
| Ibufenac (Europe) | 1975 |
| Ticrynafen | 1980 |
| Benoxaprofen | 1982 |
| Perhexilene (France) | 1985 |
| Dilevalol (Portugal and Ireland) | 1990 |
| Bromfenac | 1998 |
| Troglitazone | 2000 |
| Nefazodone (Serzone) | 2003 |
| Ximelagatran (Exanta) | 2004 |

# ACETAMINOPHEN LIVER INJURY



# MECHANISMS OF APAP LIVER INJURY

* Protein adducts
* Reactive oxygen and nitrogen species
* Mitochondrial injury
* Apoptosis

# FIALURIDINE-INDUCED MITOCHONDRIAL INJURY IN PATIENTS

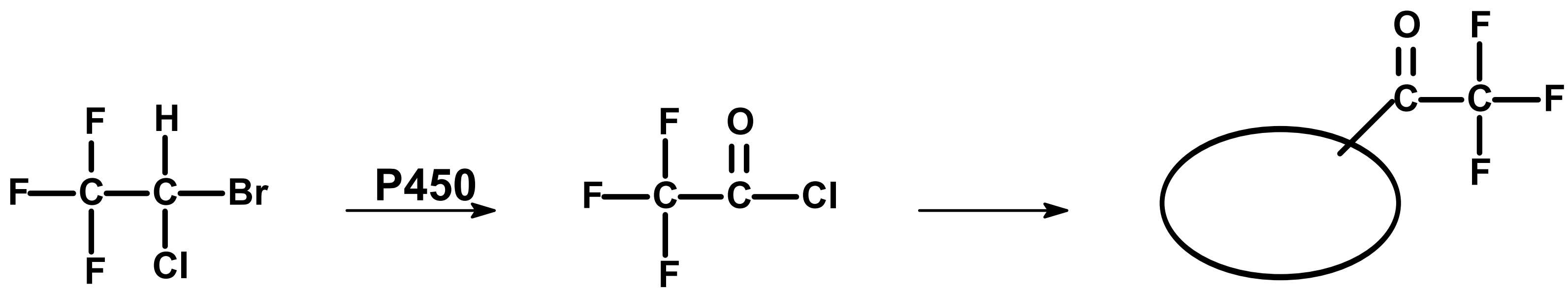
* FIAU is a uridine analog developed for hepatitis B treatment
* Administration to 15 patients resulted in 7 developing severe mitochondrial liver damage with 5 dying and 2 receiving liver transplant
* Toxicity was not predicted from rodent studies

# MECHANISM OF FIAU LIVER INJURY

- Toxicity of FIAU is apparently due to FIAU-TP which inhibits mitochondrial DNA polymerase-γ and DNA synthesis
- Humans and not rodents have human nucleoside transporter 1 (hENT1) in the mitochondrial membrane

*E.W. Lee, et al., J.Biol.Chem., 281, 16700 (2006)*

# HALOTHANE-INDUCED ALLERGIC HEPATITIS




Halothane

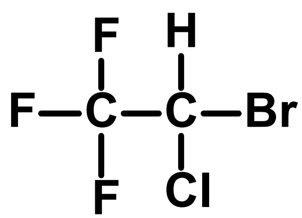
Trifluoroacetyl Chloride

Hepatocyte

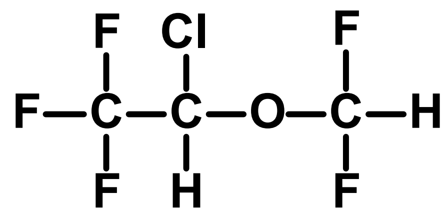
Humoral and Cellular Immune Responses

Toxicity

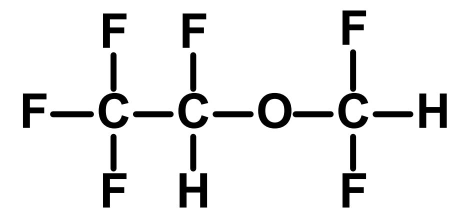
# OTHER HALOTHANE DERIVATIVES



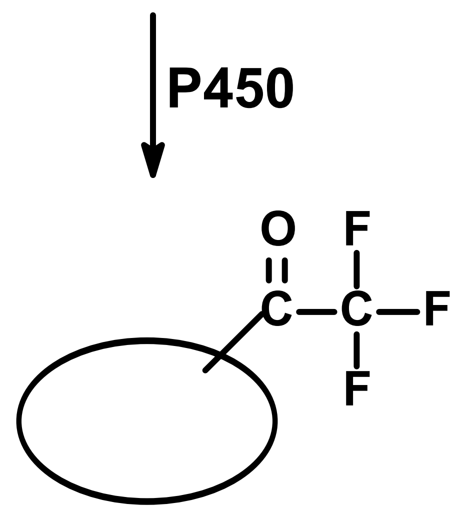
Halothane



Isoflurane



Desflurane




Hepatocyte

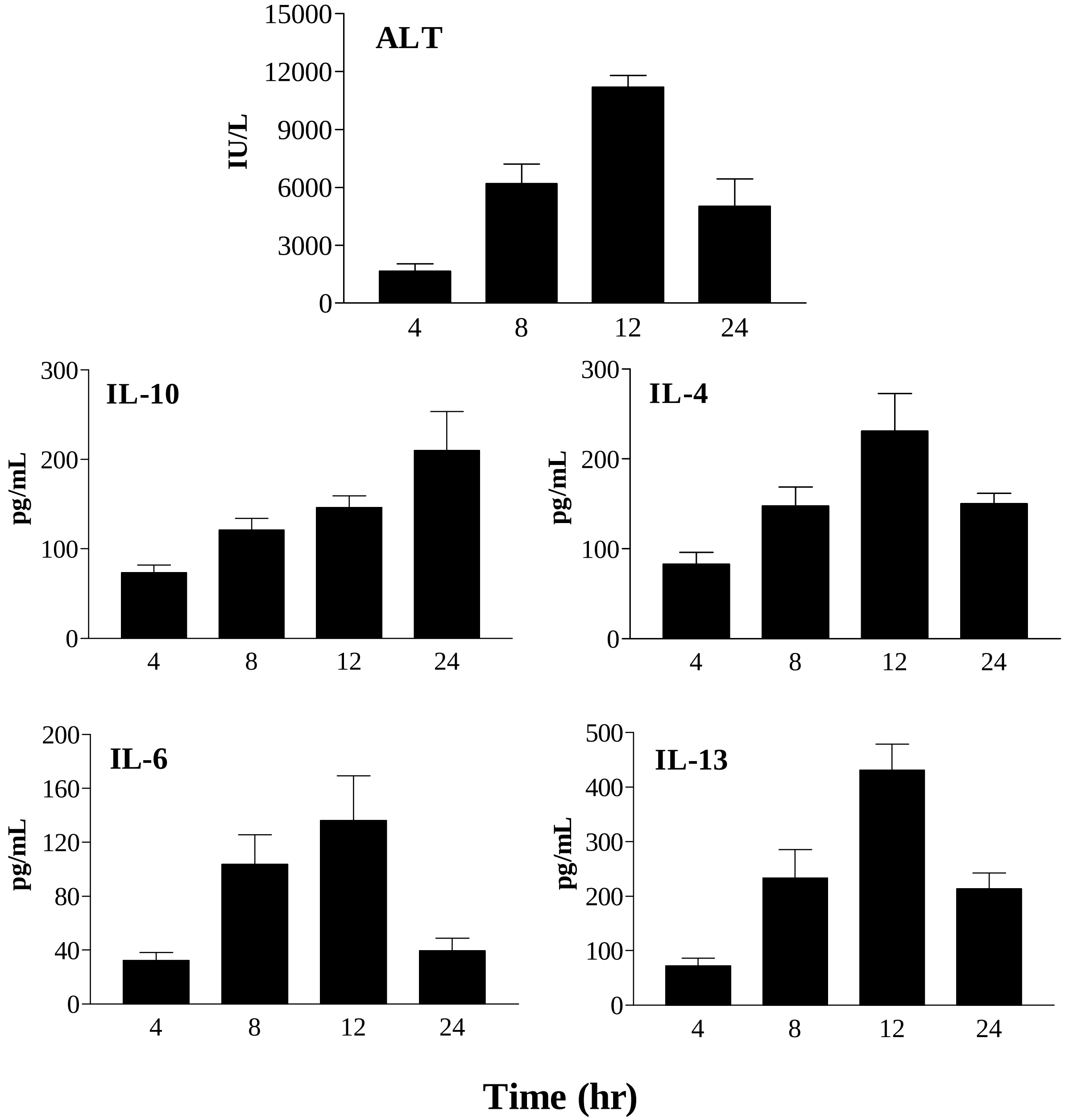
# ANTIBODIES ASSOCIATED WITH OTHER DRUGS CAUSING HEPATITIS

| Drug | Antigen |
|---|---|
| Tienilic acid | CYP2C9 |
| Dihydralazine | CYP1A2 |
| Ethanol | CYP2E1, CYP3A4, CYP2E1-hydroxy-ethyl radical |

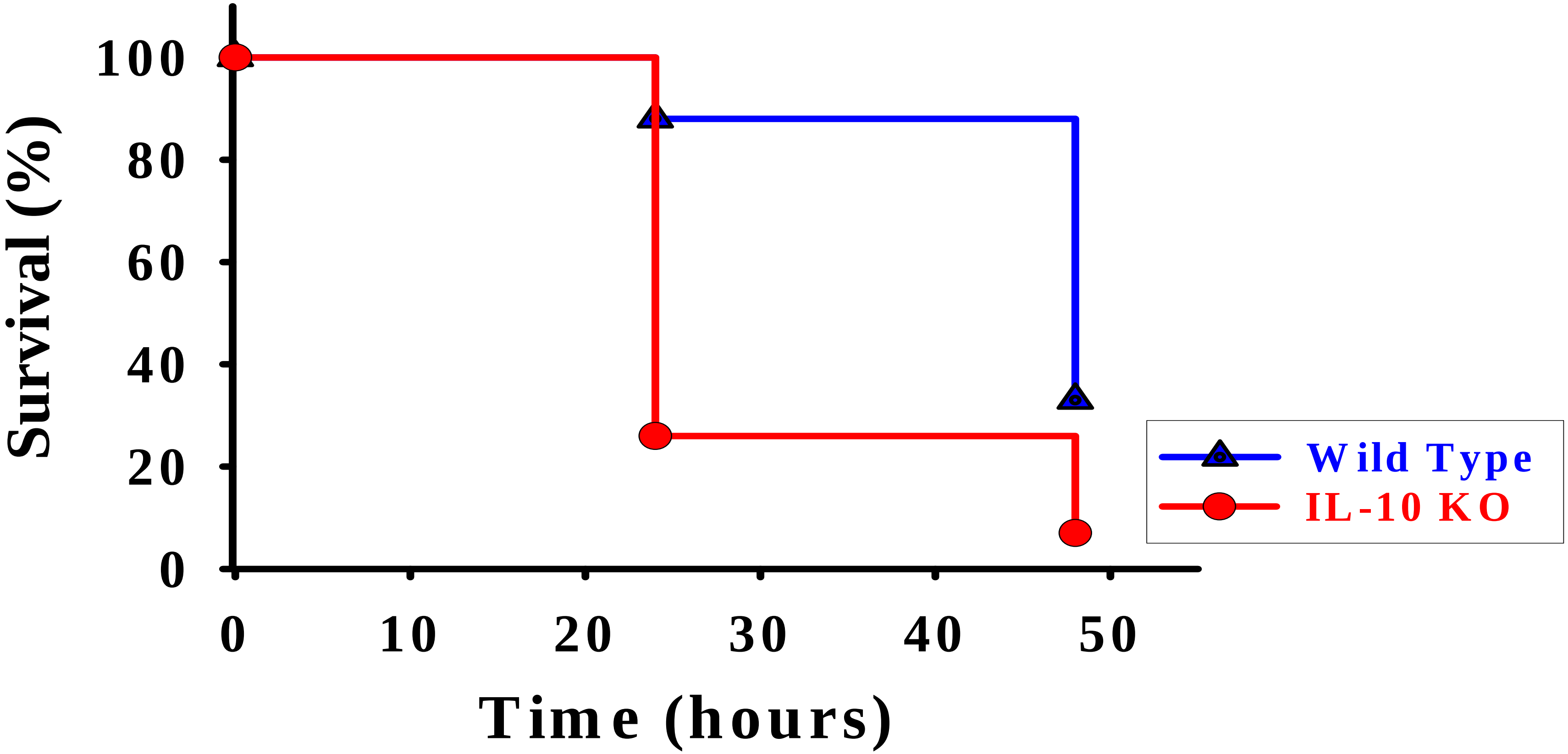
# T CELL REACTIVITY ASSOCIATED WITH DRUGS CAUSING ALLERGIC HEPATITIS

| | |
|---|---|
| Cotrimoxazole | Chlorpromazine |
| Erythromycin | Amineptine |
| Ketoconazole | Dothiepine |
| Ampicillin | Phenytoin |
| Allopurinol | Carbamazepine |
| Ibuprofen | Tamoxifen |
| Captopril | Glibenclamide |
| α-Methyldopa | Lovastatin |
| Enalapril | Propylthiouracil |

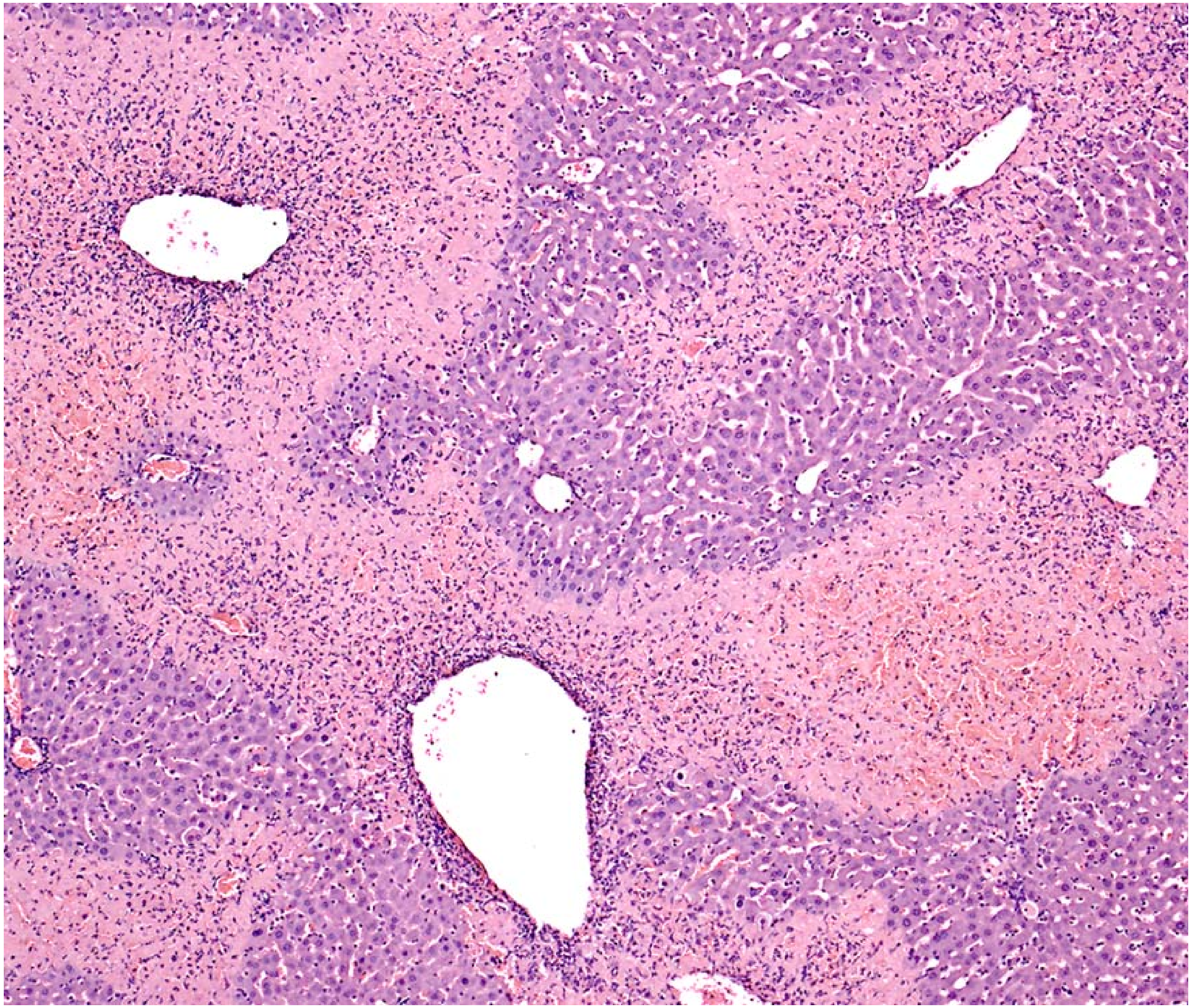
*Gut, 41, 534 (1997)*


ALT
IU/L
15000
12000
9000
6000
3000
0
4
8
12
24
IL-10
pg/mL
300
200
100
0
IL-4
IL-6
200
160
120
80
40
IL-13
500
400
Time (hr)

# IL-10 DEFICIENCY INCREASES APAP-INDUCED LIVER INJURY AND DEATH



# APAP-LIVER NECROSIS IN IL-10$^{-/-}$ MOUSE



# OTHER FACTORS INVOLVED IN AILI

**Protective:**

* IL-4, IL-6, IL-13, COX-2, Kupffer cells
* Nrf2, heme oxygenase 1

**Protoxicant:**

* IFN-γ, MIF, osteopontin, neutrophils, NK and NKT cells

# DRUG-INDUCED LIVER INJURY NETWORK (DILIN)

* Sponsored by NIDDK in 2004 to develop a registry of DILI patients
* DILIN centers at U. of North Carolina, Duke, U. of Michigan, U. of Connecticut, and U. of California in SF
* Samples are collected for biochemical, serological, and genetic testing by investigators throughout the country

## Summary

* Drug-drug interactions are a major cause of Type A ADRs, can be predictable, and lead to SADRs
* Type B ADRs are a major cause of SADRs, are highly host dependent, and may be caused by rare allelic forms of enzymes, transporters, receptors, ion channels, transcription factors, etc
* Type B ADRs also appear to be mediated by specific Abs and T cells that are induced by drug-protein adducts

# Summary

* Numerous factors likely protect most people from getting serious Type B ADRs
* Large scale genomic studies show promise in uncovering susceptibility factors that may be used to screen patients before receiving drugs.
* Mechanisms of DILI are important
* Prevent SADRs by limiting drug-protein adduct formation
* Prevent SADRs by using low doses of drugs

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